

WHAT IS CLAIMED IS:

- 1 1. A method of determining the ability of a *Mycobacterium*
2 *tuberculosis* bacterium to oxidize a thioamide or a thiocarbonyl, said method comprising
3 detecting a mutation in an EtaA gene (SEQ ID NO:1) in said bacterium, wherein
4 detection of the mutation is indicative of decreased ability to oxidize a thioamide or a
5 thiocarbonyl.
- 1 2. The method of claim 1, wherein the mutation is a frameshift
2 mutation selected from the group consisting of: a deletion at position 65, an addition at
3 position 567, and an addition at position 811.
- 1 3. The method of claim 1, wherein the mutation is a single nucleotide
2 polymorphism which causes an amino acid substitution in an amino acid sequence
3 encoded by said EtaA gene compared to an amino acid sequence of SEQ ID NO:2.
- 1 4. The method of claim 3, wherein the single nucleotide
2 polymorphism causes an amino acid substitution selected from the group consisting of:
3 G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.
- 1 5. A method of claim 1 wherein the mutation is detected by
2 (a) amplifying the EtaA gene, or a portion thereof containing the
3 mutation, with a set of primers to provide an amplified product,
4 (b) sequencing the amplified product to obtain a sequence, and
5 (c) comparing the sequence of the amplified product with the
6 sequence of a wild-type EtaA gene (SEQ ID NO:1) or portion thereof,
7 wherein a difference between the sequence of the amplified product and the sequence of
8 the wild-type EtaA gene or portion thereof indicates the presence of a mutation.
- 1 6. A method of claim 5, wherein at least one of said primers is
2 selected from the group consisting of:
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4),
5 5' ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);

8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);
9 5' TGAAGTCAGGTCGCGAAC 3' (SEQ ID NO:9);
10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);
11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);
12 5' AACCTAGCGTGTACATG 3' (SEQ ID NO:12);
13 5' TCTATTTCCCATCCAAG 3' (SEQ ID NO:13); and
14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 7. A method of claim 5, wherein the primers are
2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3), and
3 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 8. A method of claim 5, wherein said amplification is by polymerase
2 chain reaction.

1 9. A method of claim 1, wherein said mutation is detected by
2 hybridizing DNA from said bacterium to a test nucleic acid under stringent conditions.

1 10. A method of claim 9, wherein either said DNA from said bacterium
2 or said test nucleic acid is immobilized on a solid support.

1 11. A method of claim 1, wherein said mutation is detected by
2 (a) subjecting said EtaA gene to digestion by restriction enzymes,
3 (b) separating the resulting restriction products to form a pattern of
4 restriction fragment lengths, and
5 (c) comparing the pattern of restriction fragment lengths to a
6 pattern of restriction fragment lengths formed by subjecting a known EtaA gene to the
7 same restriction enzymes.

1 12. A method of claim 11, wherein said known EtaA gene is selected
2 from the group consisting of (a) a frameshift mutation consisting of a deletion at position
3 65, an addition at position 567, and an addition at position 811, and (b) a single
4 nucleotide polymorphism which causes an amino acid substitution selected from the
5 group consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

13. A method of claim 1, wherein said mutation is detected by specifically binding an antibody to a mutated product of the EtaA gene, wherein the specific binding of the antibody to the mutated gene product is indicative of a mutation which inhibits the ability of the bacterium to oxidize a thioamide.

14. A method of claim 13, wherein said gene product is in, or is isolated from, sputum.

15. A method of claim 13, wherein detection of said specific binding of said antibody and said mutated gene product is by ELISA.

16. A method of claim 1, wherein said thioamide or thiocarbonyl is selected from the group consisting of ethionamide, thiacetazone, and thiocarlide.

17. A method of claim 1, wherein said mutation is detected by

- (a) culturing said bacterium in the presence of ethionamide; and
- (b) testing for the presence or absence of (2-ethyl-pyridin-4-yl)methanol,

wherein the absence of (2-ethyl-pyridin-4-yl)methanol indicates that the bacterium has a mutation which is indicative of decreased ability to oxidize a thioamide.

18 A method of claim 17 wherein the presence or absence of (2-ethylpyridin-4-yl)methanol is tested by subjecting a medium in which the bacterium is cultured, or the bacterium, to analysis by thin-layer chromatography, high pressure liquid chromatography, or mass spectrometry.

19 A method of claim 17, wherein the ethionamide of step (a) is radioactively labeled.

20. A method of claim 17, wherein the (2-ethyl-pyridin-4-yl)methanol is radioactively labeled.

21. A method of screening an individual for a *Mycobacterium tuberculosis* bacterium resistant to treatment by a thioamide or a thiocarbonyl drug, comprising

(a) obtaining a biological sample containing said bacterium from said individual, and

6 (b) detecting a mutation in an EtaA gene (SEQ ID NO:1) in said
7 bacterium, wherein detection of the mutation is indicative said bacterium is resistant to
8 treatment by a thioamide or a thiocarbonyl drug.

1 22. A method of claim 21, wherein the mutation is detected by
2 (a) amplifying the EtaA gene with a set of primers to provide an
3 amplified product,
4 (b) sequencing the amplified product to obtain a sequence, and
5 (c) comparing the sequence of the amplified product with the
6 sequence of a wild-type EtaA gene (SEQ ID NO:1),
7 wherein a difference between the sequence of the amplified product and
8 the sequence of the wild-type EtaA gene indicates the presence of a mutation.

1 23. A method of claim 21, wherein at least one of said primers is
2 selected from the group consisting of:
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4), 5'
5 ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);
8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);
9 5' TGA ACTCAGGTCGCGAAC 3' (SEQ ID NO:9);
10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);
11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);
12 5' AACCTAGCGTGACATG 3' (SEQ ID NO:12);
13 5' TCTATTTCCCATCCAAG 3 (SEQ ID NO:13); and
14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 24. A method of claim 21, wherein said primers are
2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3) and 5'-
3 ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 25. A kit for determining the ability of a *Mycobacterium tuberculosis*
2 bacterium to oxidize a thioamide or a thiocarbonyl, the kit comprising:
3 (a) a container, and

4 (b) primers for amplifying an EtaA gene of said bacterium or a portion of
5 said EtaA gene containing a mutation affecting the ability of the bacterium to oxidize a
6 thioamide.

1 26. A kit of claim 25, wherein at least one of said primers is selected
2 from the group consisting of:
3 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3),
4 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4),
5 5' ATCATCCATCCGCAGCAC 3' (SEQ ID NO:5);
6 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:6);
7 5' GCATCGTGACGTGCTTG 3' (SEQ ID NO:7);
8 5' AAGCTGCAGGTTCAACC 3' (SEQ ID NO:8);
9 5' TGAAGTCAGGTCGCGAAC 3' (SEQ ID NO:9);
10 5' AACATCGTCGTGATCGG 3' (SEQ ID NO:10);
11 5' ATTTGTTCCGTTATCCC 3' (SEQ ID NO:11);
12 5' AACCTAGCGTGTACATG 3' (SEQ ID NO:12);
13 5' TCTATTTCCCATCCAAG 3 (SEQ ID NO:13); and
14 5' GCCATGTCGGCTTGATTG 3' (SEQ ID NO:14).

1 27. A kit of claim 25, wherein the primers are
2 5'-GGGGTACCGACATTACGTTGATAGCGTGGA-3' (SEQ ID NO:3), and
3 5'-ATAAGAATGCGGCCGCAACCGTCGCTAAAGCTAAACC-3' (SEQ ID NO:4).

1 28. A kit of claim 25, further comprising a mutated EtaA gene for use
2 as a positive control.

1 29. A kit of claim 28, wherein said mutated EtaA gene is selected from
2 the group consisting of (a) a frameshift mutation consisting of a deletion at position 65, an
3 addition at position 567, and an addition at position 811, and (b) a single nucleotide
4 polymorphism which causes an amino acid substitution selected from the group
5 consisting of: G43C, P51L, D58A, Y84D, T186K, T342K, and A381P.

1 30. A kit for determining the ability of a *Mycobacterium tuberculosis*
2 bacterium to oxidize a thioamide, the kit comprising:
3 (a) a container, and
4 (b) (2-ethyl-pyridin-4-yl)methanol.

1 31. A kit for determining the ability of a *Mycobacterium tuberculosis*
2 bacterium to oxidize a thioamide, the kit comprising:

- 3 (a) a container, and
4 (b) radiolabeled ethioamide.

1 32. A kit for determining the ability of a *Mycobacterium tuberculosis*
2 bacterium to oxidize a thioamide or thiocarbonyl, the kit comprising:

- 3 (a) a container, and
4 (b) an antibody which specifically binds to a product of a EtaA gene
5 selected from the group consisting of a wild-type EtaA gene (SEQ ID NO:1) and a
6 mutated EtaA gene.

1 33. A kit for determining the ability of a *Mycobacterium tuberculosis*
2 bacterium to oxidize a thioamide, the kit comprising:

- 3 (a) a container, and
4 (b) an antibody which specifically binds to (2-ethyl-pyridin-4-
5 yl)methanol.

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